UPF1 DUX NMD in Facioscapulohumeral Muscular Dystrophy

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Next generation sequencing has revolutionized the field of human genetics, and discovery of disease causing mutations continues at an accelerated pace. However, for some human diseases, knowledge of the genetic mutation is not sufficient to resolve disease etiology. One such example is facioscapulohumeral muscular dystrophy (FSHD), an adult-onset muscular dystrophy characterized by progressive weakness in a subset of skeletal muscle groups of the upper body, namely ones in the face, shoulders and upper arms. Previous studies showed that FSHD is caused by defective epigenetic repression of a macrosatellite array in a region of chromosome 4, which results in mis-expression of DUX4 in skeletal muscle. DUX4 is a testis-specific transcription factor that activates genes involved in germline development, but its mis-expression is toxic to muscle cells. However, how DUX4 expression is regulated in normal and pathologic states remained poorly understood.

A new Fred Hutch collaboration between the labs of Stephen Tapscott (Human Biology and Clinical Divisions) and Robert Bradley (Basic Sciences and Public Health Sciences Divisions), spearheaded by graduate student Qing Feng and published in *eLife*, describes a novel feedback loop between a quality control pathway called nonsense-mediated decay (NMD) and DUX4. "Our findings indicate that regulated RNA degradation contributes to the expression and downstream toxicity of DUX4, and also suggest a new mechanism for post-transcriptional autoregulation of transcription factors," said Dr. Bradley.

The study began by reexamining previous data from the Tapscott lab that had used deep sequencing of RNA to determine both coding and non-coding RNAs induced by high levels of DUX4 (Young et al., 2013). This analysis revealed that increased DUX4 levels resulted in increased abundance of many coding RNA isoforms with premature stop codons (nonsense mutations) upstream of splice junctions, which are predicted to be NMD substrates. To explore whether increased levels of NMD substrates were linked to reduced NMD efficiency, the authors compared relative levels of a heterologous NMD substrate (β-globin open reading frame with or without a premature stop codon) and found that DUX4-expressing myoblasts had two-fold higher levels of the β-globin NMD reporter. Globally, DUX4 expression increased levels of predicted NMD substrates for all classes of splicing events examined.

Next, the authors pursued the mechanism of NMD inhibition following DUX4 expression. Because DUX4 expression led to up-regulation, rather than down-regulation, of most NMD factors, a pattern reminiscent of UPF1 knockdown cells, the authors hypothesized that protein levels of UPF1, a central component of the NMD machinery, might be divorced from its mRNA levels. To test this, the authors measured UPF1 levels, as well as levels of NMD factors that were transcriptionally up-regulated in DUX4-expressing myoblasts (SMG7 and UPF3B) and found that only UPF1 was robustly down-regulated. The tight coupling between DUX4 protein production, decreased UPF1 levels, and increased levels of NMD substrates revealed by time-course analysis suggested that low levels of UPF1 and possibly other NMD factors, contributed to inefficient NMD in DUX4-expressing myoblasts. To test if DUX4 expression leads to UPF1 degradation, the researchers inhibited the proteasome (with MG-132) in DUX4-expressing or control myoblasts and found that UPF1 levels were restored in DUX4-expressing, but not control cells. The authors next tested whether DUX4 itself was an NMD substrate, as it contains a constitutively spliced intron within its 3' untranslated region (3' UTR). To test this, the authors knocked down UPF1 in FSHD myoblasts and then differentiated them into myotubes to induce DUX4 expression. Indeed, DUX4 mRNA expression was four-fold higher in UPF1 knockdown cells.

A characteristic feature of FSHD is the variegated nature of DUX4 expression in muscle cells, meaning that only a small percentage of FSHD nuclei are DUX4+. Intriguingly, UPF1 knockdown led to an increase in the fraction of DUX4+ nuclei (from 0.3 to 2.1%). Finally, the investigators showed that expressing DUX4 in FSHD myotubes led to a five-fold increase in the levels of endogenously transcribed DUX4 mRNA. These data led the authors to propose a double negative feedback loop where DUX4 indirectly stabilizes its own mRNA by inhibiting NMD. Overall, this study showed that impaired RNA quality control mechanisms might contribute to FSHD pathophysiology. "The next key step is to test whether these abnormalities in nonsense-mediated decay explain clinical aspects of FSHD, such as inflammation in affected muscles, that are not yet understood," concluded Dr. Bradley.

Feng Q, Snider L, Jagannathan S, Tawil R, van der Maarel SM, Tapscott SJ, Bradley RK. 2015. A feedback loop between nonsense-mediated decay and the retrogene in facioscapulohumeral muscular dystrophy. *eLife*, 4.

Young JM, Whiddon JL, Yao Z, Kasinathan B, Snider L, Geng LN, Balog J, Tawil R, van der 560 Maarel SM, Tapscott SJ. 2013. DUX4 binding to retroelements creates promoters that are 561 active in FSHD muscle and testis. *PLoS Genet* 9: e1003947.

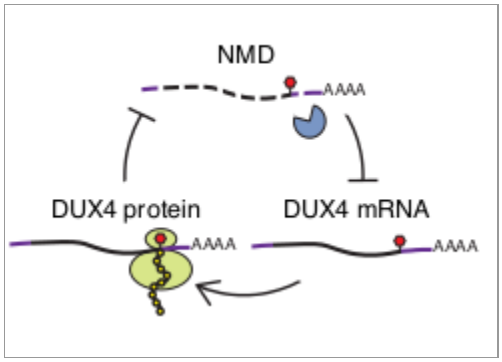

Image provided by Dr. Robert Bradley

Schematic of potential double-negative feedback loop between DUX4 and NMD, in which DUX4 inhibits NMD and NMD degrades DUX4 mRNA.